PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference TYDS:205287482	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. **PCT/AU2004/000083**	International filing date *(day/month/year)* 23 January 2004	Priority date *(day/month/year)* 24 January 2003

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] C12Q 1/68

Applicant

HUMAN GENETIC SIGNATURES PTY LTD et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of **2** sheets, as follows:
      - [X] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:

[X]	Box No. I	Basis of the report
[ ]	Box No. II	Priority
[ ]	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
[ ]	Box No. IV	Lack of unity of invention
[X]	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
[X]	Box No. VI	Certain documents cited
[ ]	Box No. VII	Certain defects in the international application
[ ]	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
7 July 2004	12 August 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer JANE MCHENRY Telephone No. (02) 6283 2091

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/AU2004/000083

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language which is the language of a translation furnished for the purposes of:

   ☐ international search (under Rules 12.3 and 23.1 (b))

   ☐ publication of the international application (under Rule 12.4)

   ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

   ☐ the international application as originally filed/furnished

   ☒ the description:

   pages **1-88** as originally filed/furnished
   pages* received by this Authority on with the letter of
   pages* received by this Authority on with the letter of

   ☒ the claims:

   pages **89 and 90** as originally filed/furnished
   pages* as amended (together with any statement) under Article 19
   pages* **92** received by this Authority on **7 July 2004** with the letter of **7 July 2004**
   pages* **91** received by this Authority on **30 July 2004** with the letter of **29 July 2004**

   ☒ the drawings:

   pages **1-11** as originally filed/furnished
   pages* received by this Authority on with the letter of
   pages* received by this Authority on with the letter of

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing (*specify*):
   ☐ any table(s) related to the sequence listing (*specify*):

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing (*specify*):
   ☐ any table(s) related to the sequence listing (*specify*):

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-32	YES
	Claims		NO
Inventive step (IS)	Claims	1-32	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-32	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents from the International Search Report is referred to in this report:

D1 = Christensen U B & Pedersen E B (2002) Nucleic Acid Res. 30(22): 4918-4925.

D2 = Robertson K D & Jones P A (2000) Carcinogenesis 21(3): 461-467.

The invention appears to reside in a method that uses intercalating nucleic acid (INA) molecules to detect methylated nucleic acids in a sample.

NOVELTY & INVENTIVE STEP

D1 discloses intercalating nucleic acid (INA) molecules. The intercalating pseudo-nucleotide, the phosphoramidite of (*S*)-1-*O*-(4,4'-dimethoxytriphenylmethyl)-3-*O*-(1-pyrenylmethyl)-glycerol, is inserted into a DNA strand to generate an INA. These INAs have a higher affinity for complementary ssDNA and ssRNA. There is no suggestion to use this INA to detect methylated nucleic acids in a sample. Claims 28-32 refer to a kit when used in the method of claims 1 to 27. Therefore claims 1-32 are novel and inventive.

D2 is a review of the DNA methylation and its effects on the mammalian genome. The major findings and future directions in this field are discussed. There is no suggestion to use INAs to detect methylated nucleic acids. Therefore claims 1-32 are novel and inventive in light of this document.

Box No. VI **Certain documents cited**

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
WO 2003/051901	26 June 2003	18 December 2002	18 December 2001
WO 2003/052132	26 June 2003	18 December 2002	18 December 2001
WO 2003/052133	26 June 2003	18 December 2002	18 December 2001
WO 2003/052134	26 June 2003	18 December 2002	18 December 2001

These documents all disclose intercalating nucleic acid (INA) molecules. However, none of these documents disclose the use of these INA's to detect methylated nucleic acid molecules. Therefore claims 1-27 are novel and inventive in light of these documents. However, claim 28-32 refer to a kit comprising an INA. These documents teach sequence specific INA molecules. Therefore these claims may not be novel or inventive in light of any one of the above documents.

The priority date of the present application appears to be valid. However, these documents may be considered relevant during National phase examination in some states.

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:
   a. type of material
      [X] a sequence listing
      [ ] table(s) related to the sequence listing
   b. format of material
      [X] in written format
      [X] in computer readable form
   c. time of filing/furnishing
      [X] contained in the international application as filed
      [X] filed together with the international application in computer readable form
      [ ] furnished subsequently to this Authority for the purposes of search and/or examination
      [ ] received by this Authority as an amendment* on
2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."

17. The method according to claim 16 wherein the capture ligand is selected from the group consisting of INA probe, PNA probe, and oligonucleotide probe.

18. The method according to claim 15 wherein both the capture ligand and the detector ligand are an INA ligand.

19. The method according to any one of claims 15 to 18 wherein the detector ligand is an INA ligand capable of distinguishing between methylated and unmethylated cytosine of DNA and the degree or amount of binding of the detector ligand is indicative of the extent of methylation of the target nucleic acid.

20. The method according to any one of claims 15 to 19 wherein the support is selected from the group consisting of plastic materials, fluorescent beads, magnetic beads, shaped particles, plates, microtiter plates, synthetic or natural membranes, latex beads, polystyrene, column supports, glass beads or slides, nanotubes, arrays, fibres, organic, and inorganic supports.

21. The method according to claim 20 wherein the support is a magnetic bead, a fluorescent bead, a shaped particle, bead array, or a microtiter plate with one or more wells.

22. The method according to any one of claims 15 to 21 wherein a plurality of capture ligands are arrayed on the solid support.

23. The method according to any one of claims 1 to 22 wherein the INA detector ligand has a detectable label attached thereto.

24. The method according to claim 23 wherein detectable label is selected from the group consisting of chemiluminescence, fluorescence, radioactivity, enzyme, hapten, and dendrimer.

25. The method according to any one of claims 1 to 24 wherein the nucleic acid bound to the INA detector ligand is further processed or treated.

26. The method according to claim 25 wherein the nucleic acid is amplified using polymerase chain reaction using primers directed to regions of nucleic acid.

27. The method according to claim 26 wherein the primers are INA ligands.

28. A kit when used in analysing nucleic acid which has been treated with an agent that modifies unmethylated cytosine according to the method of any one of claims 1 to 27 comprising at least one INA ligand capable of distinguishing between methylated and unmethylated cytosine of DNA.

29. The kit according to claim 28 wherein one or more INA ligands are immobilized to a solid support.

30. The kit according to claim 29 wherein the solid support is selected from the group consisting of plastic materials, fluorescent beads, magnetic beads, shaped particles, plates, microtiter plates, synthetic or natural membranes, latex beads, polystyrene, column supports, glass beads or slides, nanotubes, arrays, fibres, organic, and inorganic supports.

31. The kit according to any one of claims 28 to 30 further comprising primers for amplifying treated DNA.

32. The kit according to claim 31 wherein the primers are INA primers.